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Determination of hyaluronidase activity in venoms using capillary electrophoresis.

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The technique used in this study was based on the addition of a known quantity of hyaluronic acid (HA) to an aliquot of crude venom sample, followed by obtaining capillary electrophoresis profiles both immediately after the mixing and after a known period of incubation. The presence of hyaluronidase (HYASE) and the degree of its activity were determined from the change in the abundance (peak height) of intact HA at its retention time. Quantitative and/or comparative enzyme activity could also be obtained from determining the incubation time needed either to achieve a selected percentage decrease in the size of the intact HA peak or to complete the digestion process as determined by the attainment of a constant profile of the oligosaccharide end products. The detection limit was 3 x 10(-6) U/microliter HYASE, defined as the decrease of the peak height of the added standard quantity of HA (0.008 mg/ml) from the initial signal-to-noise ratio of 6 down to 2; with respect to sample size, 1.5 x 10(-8) U of HYASE could be detected in 5 nl of incubated sample. The utility of the technique was illustrated by the rapid detection of HYASE activity in HYASE standards, crude bee venom and several snake venoms, the HYASE content of which has not yet been reported, and in collected high-performance liquid chromatography-separated venom fractions.

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